Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Period: \_\_\_\_\_\_

**Cellular Respiration Lab**

Ms. Ottolini, AP Biology

**Background**

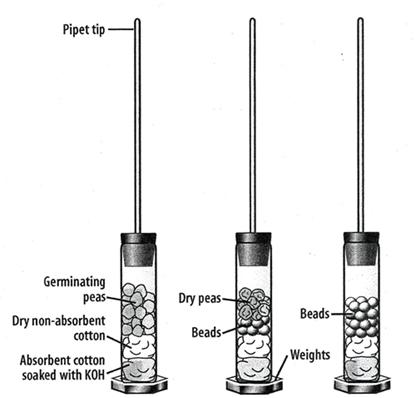
Each individual cell is responsible for the energy exchanges necessary to sustain its ordered structure.  Cells accomplish this task by breaking down nutrient molecules to generate ATP (adenosine triphosphate), which can then be used to run cellular processes that require energy.  This process is called cellular respiration which requires nutrient molecules and oxygen.   Carbon dioxide and water are products of the series of reactions involved in cellular respiration.

equation

There are several methods of indirectly measuring the rate of cellular respiration in organisms.  One method involves monitoring changes in temperature; since the process of respiration is exergonic (produces heat).  Another method is to measure either the oxygen consumption or the carbon dioxide production.  Respirometers are devices that measure these types of gas volume changes, and therefore provide information about the rate of cellular respiration.

During cellular respiration, two gases are changing in volume.  Oxygen gas is being consumed by the respiring cells and carbon dioxide gas is diffusing out of the cells.  The respirometer, therefore, has to be able to deal with two simultaneously changing gas volumes.  This is accomplished by introducing potassium hydroxide into the device.  KOH absorbs carbon dioxide, following this equation

CO2 + 2KOH --> K2CO3 + H2O

Potassium carbonate ( K2CO3 ) is a solid precipitate.  Any CO2 produced is immediately converted from a gas to a solid and is therefore no longer governed by gas laws.  This allows the respirometer to measure only one variable, the ****consumption of oxygen gas by living cells.

Two sets of three respirometers will be assembled during this lab exercise.  Each set will be incubated at a different temperature.  One respirometer will contain germinated seeds, one will contain a mix of nongerminating seeds and plastic beads, and a third will contain only plastic beads.

The purpose of the beads is to ensure that each respirometer is uniform in volume.  The respirometers will also contain a layer of cotton that has been saturated with KOH so that carbon dioxide will be absorbed.   The respirometers will be submerged in a pan of water;  water will flow from an area of high pressure to an area of low pressure.  As oxygen is used up by the respiring seeds, the gas pressure inside the respirometer will decrease and the water will flow into the pipet down its pressure gradient.

**Your Hypothesis:**

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Materials**:   25 germinating pea seeds,  25 dry seeds,  50 plastic beads,  3 respirometer vials, Weights for vials, 3 stoppers,  1 ml graduated pipets, cotton balls, water bath, ice, 100 ml graduated cylinder, thermometer, masking tape, , water, dropper bottle of 15% KOH

Safety – wear safety goggles.  KOH is caustic, avoid direct skin contact.  If contact occurs, flush affected area with running water for 10 minutes.

**Procedure:**

1.  Label three paper towels as follows:  1, 2, 3. These numbers will correspond to the respirometers of the same numbers.

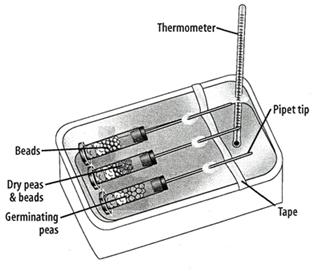
2.  Fill a graduated cylinder with 20 ml of water.  Count out 25 germinating seeds and place them into the graduated cylinder.   Record the total volume of the seeds and water in the data table.  Subtract the initial 20 ml to determine the total volume of the germinating seeds.  Pour out the water from the graduated cylinder and place the 25 germinating seeds on paper towel 1.

3.  Fill the graduated cylinder with 20ml of water.  Count out 25 dry (nongerminating) seeds and place them into the water.  Drop plastic beads into the cylinder until the final volume is the same as from step 3.

4. Place the pea/bead mixture on paper towel 2.  (Your goal here is to make sure each respirometer has the same volume).

5.  Fill the graduated cylinder with 20 ml of water.  Add beads to the water until the total volume equals the final volume from steps 2 & 3.  Place the beads on paper towel 3.

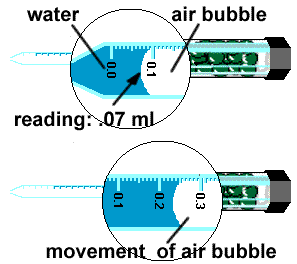
6.  Assemble the respirometers.  Begin with 3 vials , rubber stoppers and 1 ML pipets.  You will also need masking tape to wrap around the pipet to ensure that there is a tight seal inside the rubber stopper

****7.  Insert the **nontapered** end of one pipet into the upper surface of one of the rubber stoppers.  It should fit tightly.

8.  Place a piece of cotton in the bottom of each of the weighted vials.  Push the cotton firmly into the bottom of the vial with a stirring rod.  Saturate the cotton in the vial with a three drops of 15% KOH. Place another cotton ball on top.

9.  Add the peas, peas/beads, and beads to the appropriate respirometer.  Place the stoppers on each of the vials and ensure they are secured tightly.  Any leaks will cause the experiment to fail.

10.  Place a strip of masking tape across the narrow width of the water bath, approximately 2/3 of the way from one end (see diagram

11.  Place respirometers 1, 2, 3 into water bath so that the pipets rest on the masking tape prop. Your group will be assigned either the room temperature water bath or the cold water bath.  Begin time for a total of 7 minutes – this is the equilibration period, where your respirometers will become the same temperature as the water.       
**Use a thermometer to determine the water temperature:  \_\_\_\_\_\_\_\_\_**

12. At the end of 7 minutes, GENTLY submerge each of the tubes entirely in the water bath.  Some water will enter the tip of the pipet, but the influx of water should stop fairly quickly.  If it does not stop, check the respirometer for leaks.

13.  At this point, check to make sure you can read the pipets.

The air bubble should extend from the main chamber up the tube of the pipet.  The pipet may need to be rotated so that you can see the numbers.

14.  If your respirometers float, you may need to weight them with the metal washers.

15.    Record the water level in each pipet onto the data table at the Time Interval 0.

16.  Record the position of the water in each pipet at the end of 5, 10, and 15, 20 min on Data Table 2.

**Methods Summary Chart:**

|  |  |
| --- | --- |
| Independent Variable |  |
| Levels of the Independent Variable (if applicable) |  |
| Dependent Variable |  |
| Method for Measuring Changes in the Dependent Variable |  |
| Control Group (if applicable) |  |
| Constants (factors that stay the same between your control group and your experimental groups) |  |
| Number of Trials |  |

|  |  |  |  |
| --- | --- | --- | --- |
| **DATA TABLE 1: Calculation of Volume in Respirometers** | | | |
|  | Respirometer 1 (germinating seeds) | Respirometer 2 (non germinating) | Respirometer 3a (beads only) |
| Initial Volume (mL) | 20 | 20 | 20 |
| Final Volume (mL) |  |  |  |
| Volume of beads/seeds |  |  |  |

**Data Tables: Individual Lab Group**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Data Table 2: Calculation of Oxygen Consumption** | | | | | |
| Respirometer 1: Germinating Peas | | | | | |
| Time interval (min): | **0 min** | **5 min** | **10min** | **15 min** | **20 min** |
| Reading, mL |  |  |  |  |  |
| Δ  Volume, mL (reading – time 0) | N/A |  |  |  |  |
| Respirometer 2: Dry Pea Seeds | | | | | |
| Reading, mL |  |  |  |  |  |
| Δ  Volume, mL (reading – time 0) | N/A |  |  |  |  |
| Respirometer 3: Beads Only | | | | | |
| Reading, mL |  |  |  |  |  |
| Δ  Volume, mL (reading – time 0) | N/A |  |  |  |  |

**Data Tables: Class Averages**

**Graph:  Rate of Oxygen Consumption in Respirometers  (room temperature)**

Plot the independent variable on the X axis (time), and the dependent variable on the Y axis (reading on pipet).

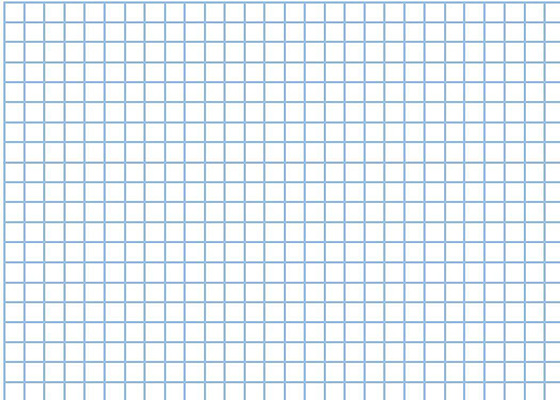
Graph a line for:

Germinating Peas (room temp)  
Germinating Peas (cold)

Non Germinating peas (room temp)  
Non Germinating peas (cold)

Beads (room temp)

\*\*Use a line of BEST FIT so that you can calculate slope\*\*



**Discussion / Conclusion Questions:**

1.   State a hypothesis that relates to temperature that is being tested by this lab exercise.

2.   State a hypothesis that relates to the state of seed germination that is being tested by this lab exercise.

3.  Calculate the RATE of oxygen consumption for the germinating seeds in both cold and room temperature water.  Rate can be calculated by determining the SLOPE of the line from your graph above.

4.  In this lab exercise, what is the purpose of the ….

1. Beads
2. KOH
3. Respirometer

5.  Explain why the water moved within the pipet.